PATENT
DOCKET NO.: 2026-4253US3

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s) : Cieplak, W. Group Art Unit: 1814

Serial No. : 08/483,326 Examiner: Bugaisky, G.

Filed : June 7, 1995

For : PERTUSSIS TOXIN GENE: CLONING AND EXPRESSION

DECLARATION UNDER 37 C.F.R. §1.131

COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

Sir:

I, Witold Cieplak, Jr., am named as the inventor in the above indicated patent application, and I state as follows:

1. In a Declaration dated March 24, 1997, I stated that prior to July 1, 1988, the claimed invention was conceived and reduced to practice. In fact, the invention was conceived and reduced to practice even before September 1, 1987. The results of these first experiments showing the invention are described below.

2. The cloned gene and its expression product have the laboratory designation mutant 4-1. Mutant 4-1 possesses and exhibits the characteristics disclosed in Patent applications 07/311,612 and its continuation 07/542,149.

3. Exhibit pages 1-3 include laboratory notebook pages which demonstrate ADP-ribosyltransferase assays involving various pertussis toxin mutants, including a demonstration of substantially reduced enzyme activity associated with mutant 4-

1. On the bottom of page 1, a brief outline of the ADP-ribosyltransferase assay is provided. The samples were incubated in the presence of the acceptor G protein transducin [*adenylate*-^{32}P]NAD$^+$ for 30 minutes at 37°C. The ADP-ribosyltransferase activity was measured as the extent of transfer of ^{32}P from the radiolabeled NAD$^+$ to transducin. The amount of ^{32}P incorporation into transducin was determined in two ways. First, the reaction samples were incubated with trichloroacetic acid (TCA) after the addition of bovine serum albumin to precipitate the proteins. The resultant TCA pellets were air dried after an ether wash and the amount of radioactivity in each pellet was determined by Cerenkov spectrometry to provide a quantitative estimate of ADP-ribosyltransferase activity. This assay revealed the lack of detectable transferase activity in the 4-1 mutant sample (labelled 4-1 on right side of table, labelled SAM #24 on left) compared to the other mutants and the positive control (labelled "PTX" on right side of table, labelled SAM #2 on left side). Second, the TCA precipitated proteins were solubilized in electrophoresis sample buffer and separated by sodium dodecylsulfate polyacrylamide gel electrophoresis. The gel was dried on filter paper and exposed to X-ray film. Page three is a copy of the resultant autoradiograph, showing that the reaction mixture containing the 4-1 mutant (fourth lane from the left) contained little or no detectable radiolabelled transducin (as evidenced by the lack of a band corresponding to 39 kDa) when compared to reaction mixtures containing other mutants or 6A-4, a wild type version of the S1 subunit. This assay confirmed the results of the quantitative analysis described above and demonstrates that mutant 4-1 has substantially reduced ADP-ribososyltransferase activity when compared to either pertussis toxin or other mutants.

4. Exhibit pages 4-5 show a stained protein gel and three Western blots which demonstrate the reactivity of mutant 4-1 with a monoclonal antibody called "SATO" (also known as 1B7). The protein gel (bottom half of page 4) shows the presence of protein in all of the samples, while the Western blots demonstrate the selective recognition of the antibodies used. The blots labelled "RαPTX" represent the protein samples seen in the protein gel, as reacted with a rabbit anti-pertussis antibody, called "RαPTX". This antibody was a polyclonal antibody which reacted with both PTX (control) and the 4-1 mutant (compare right-most lane and left-most lane). Similarly, the protein samples were reacted with the SATO monoclonal antibody, as seen in the blot labelled "SATO" on the top half of page 5. In these samples, the antibody reacted with both PTX (control) and the 4-1 mutant (compare right-most lane and left-most lane). These pages provided the first data demonstrating reactivity of the 4-1 mutant with a protective monoclonal antibody.

5. The actual dates on laboratory notebook pages described in section 2-4 above have been blocked out. I state that each laboratory notebook page in section 2-4 above was dated prior to September 1, 1987.

6. The work corresponding to section 2-4 above was carried out by me or a technician working under my direction in the United States.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the

United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 4-21-99

By: /Witold Cieplak/
Dr. Witold Cieplak, Jr.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No. 40399/177/NIHD

In re patent application of

Jerry M. Keith

Serial No. 07/542,149 Group Art Unit: 1814

Filed: June 22, 1990 Examiner: G. Bugaisky

For: PERTUSSIS TOXIN GENE:
CLONING AND EXPRESSION

DECLARATION OF WITOLD CIEPLAK, JR.

The Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Sir:

I, Witold Cieplak, Jr. hereby declare that:

(1) I have read the declaration of Dr. Jerry Keith attached hereto as Appendix 1.

(2) The copies of notebook pages attached to that declaration are copies of pages from my own notebook, as I was the one who carried out the work recorded on those pages.

(3) I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent resulting therefrom.

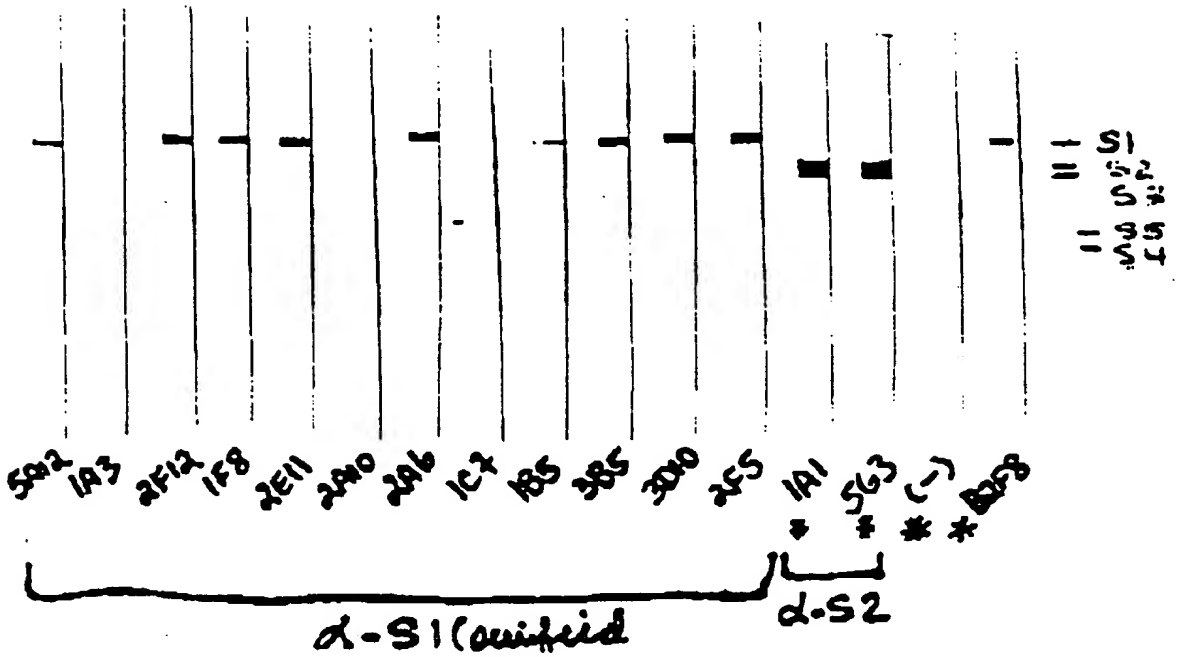
3/29/93
Date

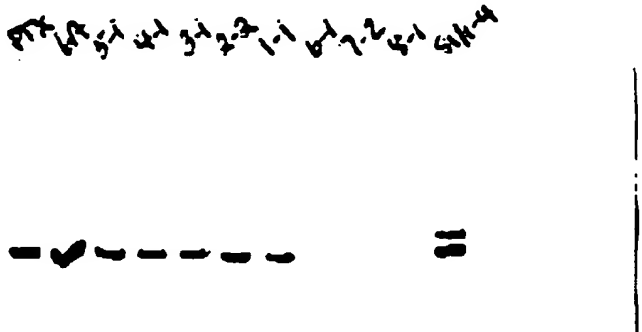
Witold Cieplak, Jr.

EXHIBIT PAGE #2

PTx
10ug

[redacted]





Amgen mutant S1 proteins
monoclonal 1B7

[redacted]

EXHIBIT PAGE #3

Take samples (Amgen) - dilute 1:1 w/ [illegible]
Run ~20ul for protein (5ug);
~10ul for western (2.5ug total);

Protein | Western

Lane
1 Blank | Blank
2 10ul [illegible] Std | Rel Std (5ul)
3 PTx (5ul) .5ug | PTx (2.5ul)
4 6B
5 5-1
6 4-1
7 3-1 — 15ul
8 2-1 — ~~20ul~~ → Same except use 10ul
9 1-1
10 8-1
11 7-2
12 10-1
13 6B-2
14
15 Blank

4

187

EXHIBIT PAGE #4

Protein Gel - Dragon mutants in mouse bone
- 10 uL of 560 ug/ml in 1x Laemmli
ie 5 ug ea (Sent Fri AM).

Next time cut down on 1-1 gel for material;

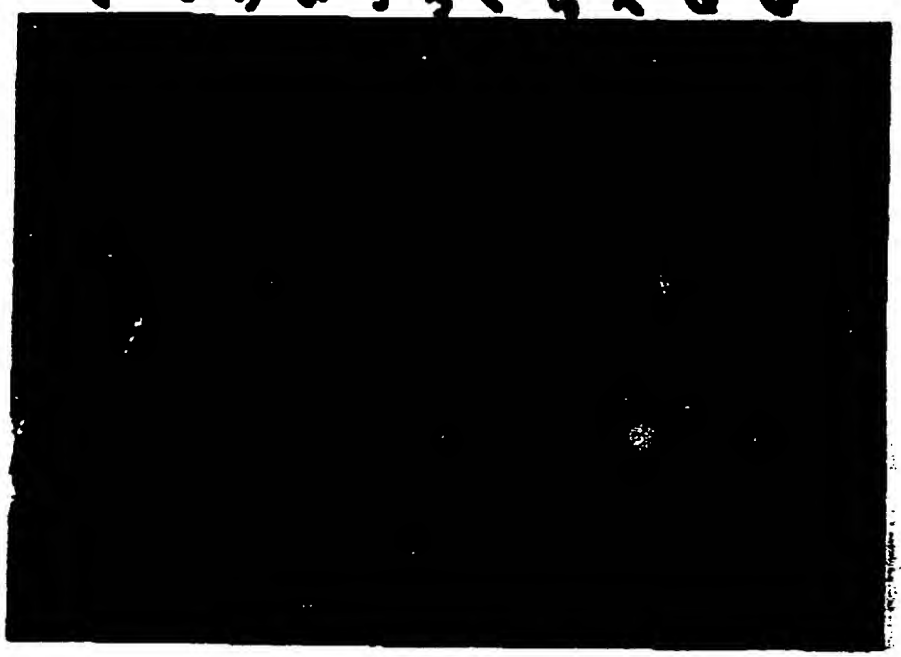


EXHIBIT PAGE #5

also [redacted]

For Western B (w/ new monoclone) and Protein

FOR 200ul of 1mg/ml in Laemmli

DTx (1ug)	Sample	2X	1X	Need
pUC18	52.2	52.2	95.5	78.4 78.4 43
~~S13~~	~~28.4~~	~~28.4~~	~~142~~	—remake
~~His 28~~	33.7	33.7	132.5	too low
Lys 9	39.6	39.6	120.7	↓
His 9	51.5	51.5	96.9	actually
Ala 9	58.1	58.1	83.7	0.36
Del 9	56.6	56.6	26.8	amu
His 8:9	48.0	48.0	103.8	
Lys 58	44.5	44.5	110.9	
Gly 41	33.1	33.1	133.7	
Ser 41	27.2	27.2	145.5	
Del 41	33.3	33.3	133.3	

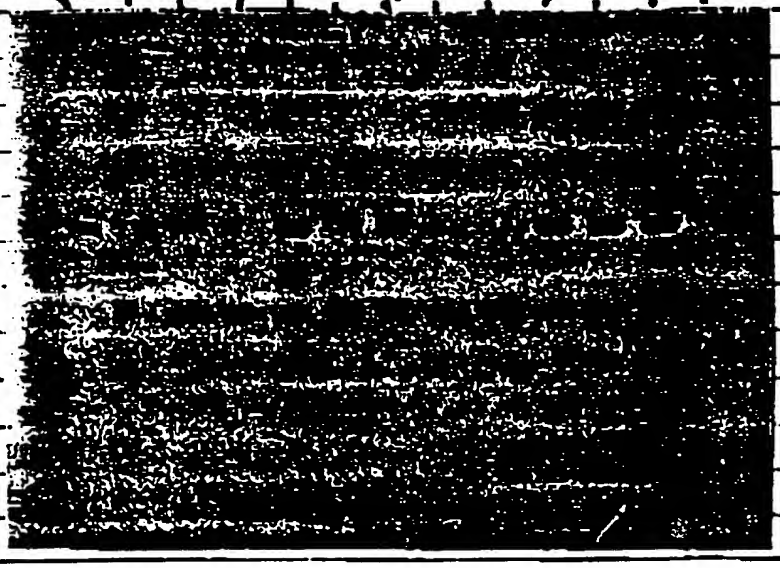
Made 200ul samples of (ea) at 1mg/ml in Laemmli;
loaded (20ul = 20ug) into each lane of gel —
1 stain
1 blot,

(Forgot about pDTx13 — minimount)

	8umole	2x	1x
remade: New pS13 —	28.43	78.43	43.1

Blotted 2 gels — probed w/ 2F12 and 1B7

EXHIBIT PAGE #6



1B7

2F12←

[redacted]

EXHIBIT PAGE #7

ADP-ribosyltransferase Assays - Arginine mutants - and S13 mutants, 4ug new transducin (glycerol)

10ug/20ul

	Stock (S1)	FOR Sup/ml	buffer
[illegible] S1	400	12.5	987.5
6A	225	22.2	977.8
7-1	218	22.9	977.1
2-2	239	20.9	979.1
3-1	267	18.7	981.3
4-1	247	20.2	979.8
5-1	156	32.0	968
6-1	126	39.7	960.3
7-2	183	27.3	972.7
8-1	135	37.0	963
20-A	230 (total)	30.0	970
S1/1-4	199	25.1	975
pUC	1.34	0.373	0.127
S13	2.55	0.196	0.304
lys 58	4.49	0.111	0.389
His 8.9	4.16	0.120	0.380
del 9	2.31	0.216	0.284
his 9	3.88	0.128	0.372
ala 9	3.44	0.145	0.355

20ug/20ul

0.5mg/1mg/ml

0.5 hr, 37°C

* [illegible] = prepared from new [illegible] - [illegible] - no glycerol;

EXHIBIT PAGE # 8

[redacted]

	365.70	4[illegible]	[illegible]	10.00	
	[illegible]	[illegible]	[illegible]	[illegible]	
	[illegible]	[illegible]	20702.80	10.00	
4	427.40	5544.60	24382.60	10.00	
5	450.30	5594.30	25768.10	10.00	2 [illegible] ✓
6	449.30	5592.00	26200.70	10.00	
7	130.70	1877.50	5925.50	10.00	
8	163.60	2097.50	8630.70	10.00	3 [illegible] ✓
9	137.90	1565.50	7625.40	10.00	
10	367.60	5671.80	20435.10	10.00	
11	396.20	5078.90	24578.20	10.00	4 [illegible] ✓
12	371.60	5389.20	21945.60	10.00	
13	196.10	2095.20	12523.20	10.00	
14	287.20	4389.90	15388.10	10.00	5 3-1
15	234.30	3437.70	12737.90	10.00	
16	34.20	211.90	748.00	10.00	
17	32.90	187.00	754.60	10.00	6 4-1 ✓
18	35.00	223.20	762.40	10.00	
19	400.60	4613.40	27887.60	10.00	
20	379.10	4982.40	25621.90	10.00	7 5-1 ✓
21	446.20	6774.50	27576.10	10.00	
22	38.40	228.60	896.20	10.00	
23	31.40	219.80	749.50	10.00	8 6 ✓
24	31.70	178.80	649.30	10.00	
25	33.30	170.70	729.40	10.00	
26	32.00	178.20	745.60	10.00	9 7 ✓
27	33.10	184.70	787.40	10.00	
28	31.90	165.30	696.20	10.00	
29	39.00	268.20	1092.50	10.00	10 8 ✓
30	35.30	208.60	892.40	10.00	
31	35.50	218.30	918.40	10.00	recount 792
32	[illegible]	[illegible]	[illegible]	[illegible]	1120A →
33	35.20	185.20	809.90	10.00	
34	37.70	257.30	947.30	10.00	
35			348		
36			963		

EXHIBIT PAGE #9

[redacted]

Construct (100mg)	cpm ± SD *	upper limit
6A	25450 ± 950	26400
1-1	7393 ± 1367	8760
2-2	22319 ± 3096	24415
3-1	13549 ± 1596	~~14~~ 15145
4-1	754 ± 7	761
5-1	26361 ± 1321	27682
6-1	764 ± 124	868
7-2	753 ± 30	783
8-1	926 ± 205	1131
first 20A	839 ± 68	907
rS1/1-4	952 ± 9	961

* S.A. of 32P-NAO may have been a little on the low side.

EXHIBIT PAGE #. 10

[redacted]

ADP-Ribosyltransferase Activity

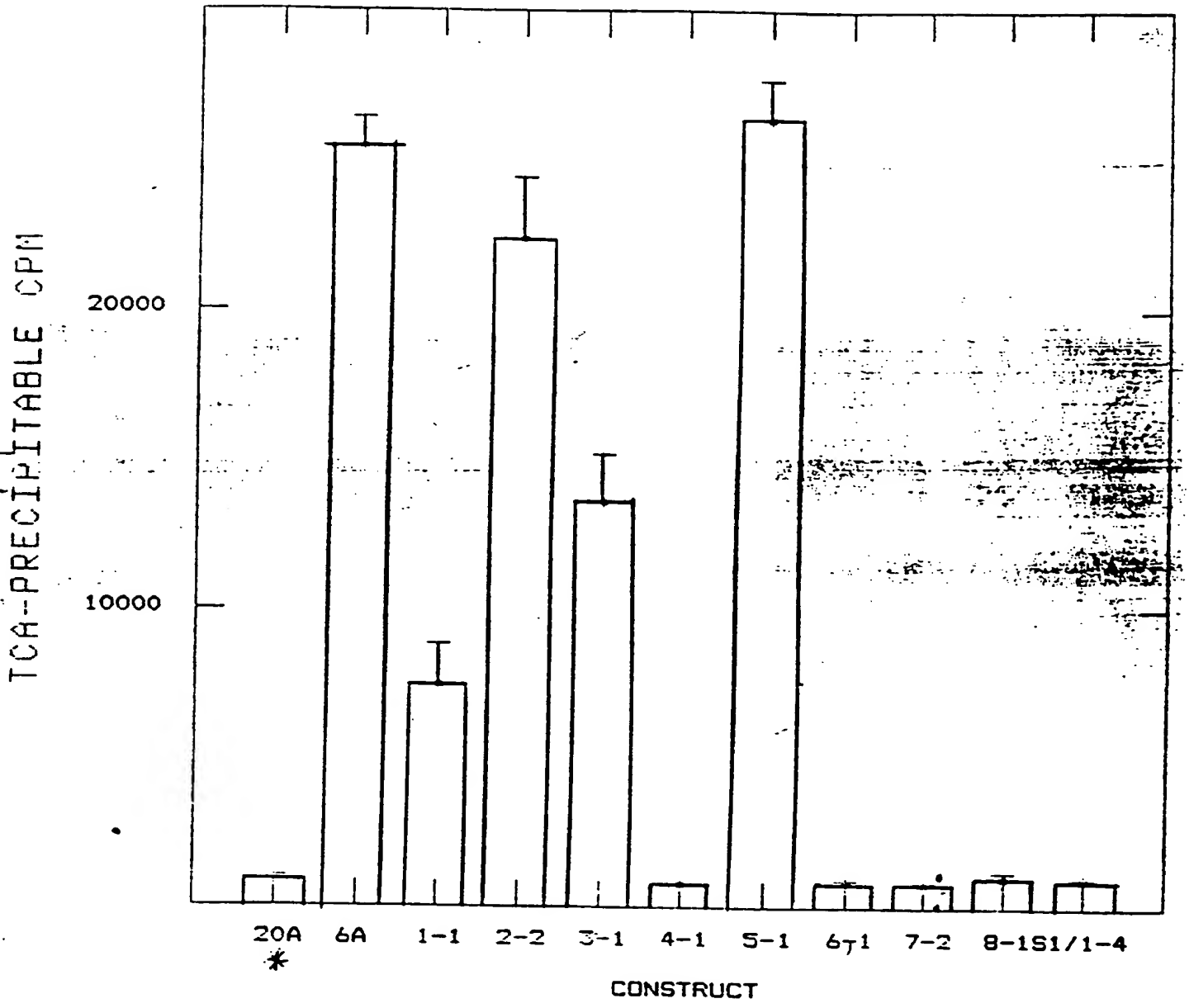
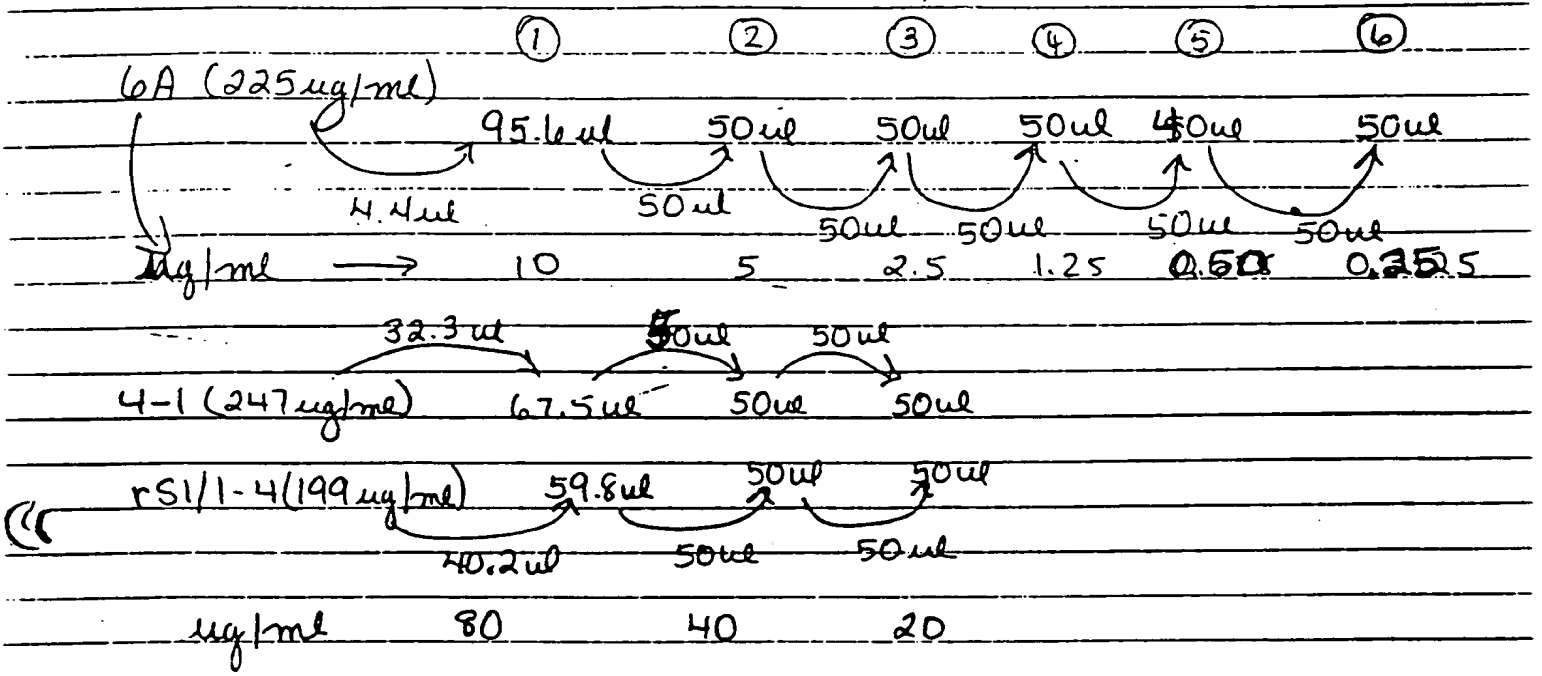


EXHIBIT PAGE #11

ADP-ribosyl transferase activity: 2A, 6A, 4-1 and S1/1-4

1) All stocks frozen in 50 mM Tris HCl, pH 8.0 protein and densitometric scans already performed

2) Dilutions for assay: use Tris buffer:



3) Use 20 ul of each prep in 40 ul assay; assay 30' at 37°C w/ 4 ug/

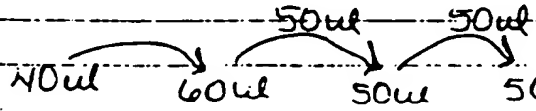
4) Final concentrations: (ug/ml)

6A 0.125, 0.250, 0.625, 1.25, 2.5, 5.

4-1 } 10, 20, 40
rS1/1-4 }

5) Reaction mixtures: 20 ul dilution
10 ul 4X cocktail
10 ul Translucin (400 ug/ml 50% glycerol)

N.B. 20A dilute



ISC

[illegible] Exhibit PAGE # 12

[redacted]

[illegible] BA[illegible]
PLA[illegible] REPEAT: [illegible] REPEAT: [illegible]
[illegible] BACK [illegible] COUNT [illegible]
CHANNEL [illegible] 2SIGMA: 2.00 BKG [illegible] BKG 2SIG: 0.00 LSR:
CHANNEL [illegible] 2SIGMA: 2.00 BKG [illegible] BKG 2SIG: 0.00 LSR:
CHANNEL [illegible] 2SIGMA: 2.00 BKG [illegible] BKG 2SIG: 0.00 LSR:
DATA CALC: CPM, UNKNOWN: REPLICATES: [illegible] NORM FACTOR: 1.00000
HALF LIFE(DAYS): [illegible]

SAM	CPM1	CPM2	CPM3	TIME	
1	28.00	159.00	574.00	[illegible]	Blank (66)
2	31.00	156.00	358.00	[illegible]	
3	754.00	11593.00	47197.00	[illegible]	6A 200ng 45609
4	759.00	10019.00	[illegible]	1.00	
5	723.00	12202.00	39958.00	1.00	" 100ng 39883
6	728.00	3558.00	42031.00	1.00	
7	[illegible]	[illegible]	35608.00	1.00	" 50ng 37581
8	755.00	11763.00	40786.00	1.00	
9	[illegible]	4358.00	21631.00	1.00	" 25ng 26686
10	570.00	9409.00	32971.00	1.00	
11	256.00	3685.00	14666.00	1.00	" 10ng 13464
12	177.00	2114.00	13494.00	1.00	
13	168.00	1974.00	9214.00	1.00	" 5ng 7588
14	154.00	1863.00	7195.00	1.00	
15	33.00	151.00	664.00	1.00	20A 1.6ug 39
16	32.00	169.00	646.00	1.00	
17	32.00	190.00	713.00	1.00	" 0.8ug 92.5
18	37.00	144.00	598.00	1.00	
19	38.00	151.00	711.00	1.00	" 0.4ug 102.5
20	39.00	191.00	725.00	1.00	
21	45.00	194.00	[illegible]	[illegible]	4-1 1.6ug 149.5
22	40.00	205.00	[illegible]	[illegible]	
23	45.00	241.00	[illegible]	[illegible]	" 0.8ug 219
24	[illegible]	203.00	740.00	[illegible]	
25	35.00	155.00	572.00	[illegible]	" 0.4ug 62.5
26	[illegible]	200.00	[illegible]	[illegible]	
27	[illegible]	410.00	1464.00	[illegible]	S1/1-4 1.6ug 2[illegible]8
28	[illegible]	466.00	[illegible]	[illegible]	
29	[illegible]	253.00	[illegible]	[illegible]	" 0.8ug 406.5 →
30	[illegible]	245.00	[illegible]	[illegible]	
31	[illegible]	241.00	534.00	[illegible]	" 0.4ug 312
32	[illegible]	215.00	502.00	[illegible]	

20A: $\bar{X} = 78$

4-1: compared to buffer

% control

4-1 max = 0.01 % > [illegible]-old [illegible] max

S1/1-4 = 0.33 % > 2,000 fold decrease

EXHIBIT PAGE # 13

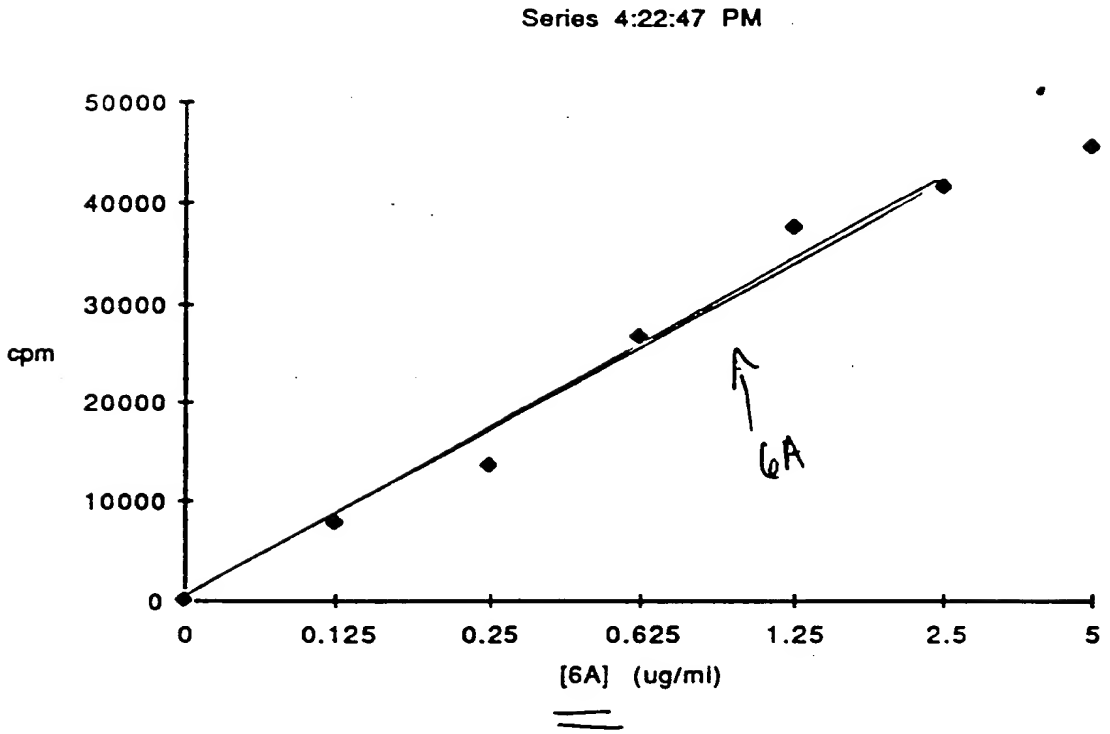
[redacted]

Sample	Final [ug/ml]	$\bar{x}$ cpm	Net cpm	
Buffer	~~0.0~~ -	616	-	
20A	0.125	8205	7589	
"	0.250	14080	13464	*
"	0.625	27301	26685	
"	1.25	38197	37581	
"	2.5	42150	41534	
"	5.0	46265	45649	
20A	10.0 equiv	~~758~~	~~-39~~	
"	20.0 eq	708	~~-89~~	78 cpm net.
"	40 equiv	655	~~-142~~	

				ved factor
H-1	10	679	-39	>5000
"	20	835	127	8481
"	40	765	110	>10,000
S1/1-4	10	~~928~~ 928	210	2564
"	20	~~1023~~ 1023	315	3419
"	40	~~928~~ 1584	929	2318

Here refer to processed protein content

EXHIBIT PAGE # 14



DP-Ribosylation of Tr... ...in

[redacted]

EXHIBIT PAGE #15

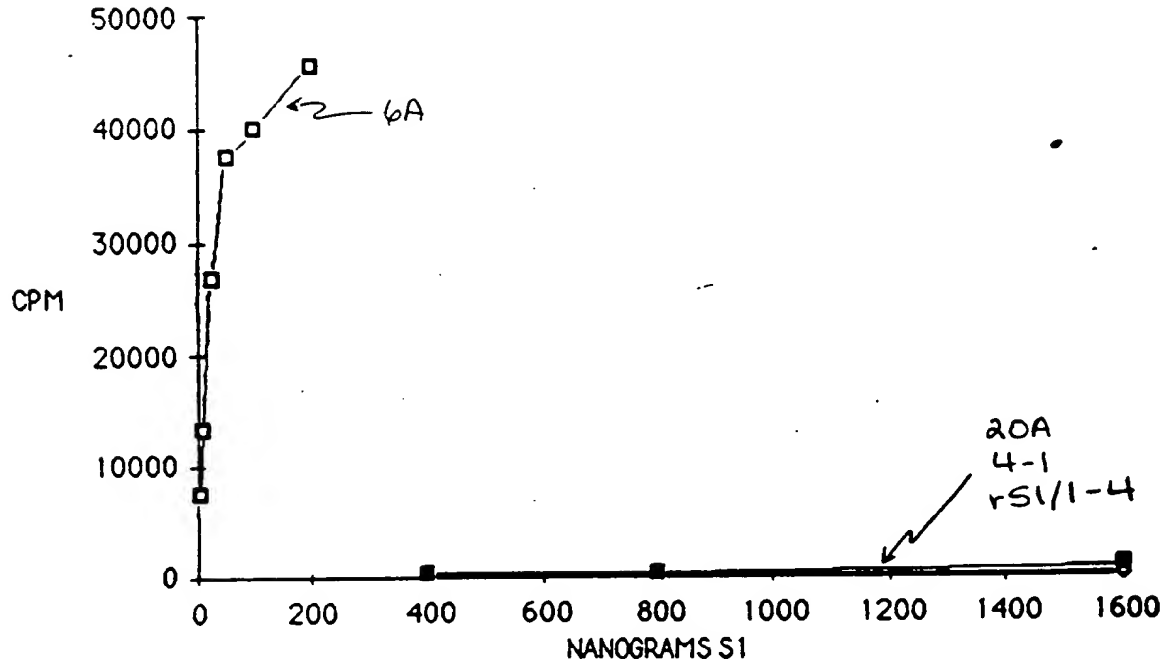


EXHIBIT PAGE # 10

VAD glycohydrolase Assay

~~for 40 ug/ml in 100 ul~~

6A	225	17.7	82.2
35A	69	57.9	42.1
39A	88	45.4	54.5
33B	113	35.3	64.7
2B	125	32.0	68.0
3B	157	25.4	74.6
1-1	75	53.3	46.6
2-2	75		
3-1	75		
4-1	75		
5-1	75		
6-1	75		
7-2	75		
8-1	75		
20A	75?		

Assayed standard fractions in triplicate ; 30°C for 4 hrs

50 ug/ml = 1

cpm x 1.5 x 1.5 ÷ 120 ÷ 76.9 = 1 pmole

EXHIBIT PAGE # 11

NAD glyceraldehyde-3-phosphate dehydrogenase - used 20A glycol control all at lysosomes

	% control ± S.D. (+dH)
6A	100
35A	105.7 ± 7.6
39A	35.3 ± 2.3
33B	3.9 ± 0.8
2B	1.6 ± 1.5
3B	1.5 ± 1.2
1-1	6.1 ± 0.98
2-2	47.6 ± 3.1
3-1	9.1 ± 2.0
4-1	2.2 ± 0.4
5-1	132.1 ± 7.4
6-1	1.7 ± 0.4
7-2	2.2 ± 0.6
8-1	2.6 ± 0.4

New data

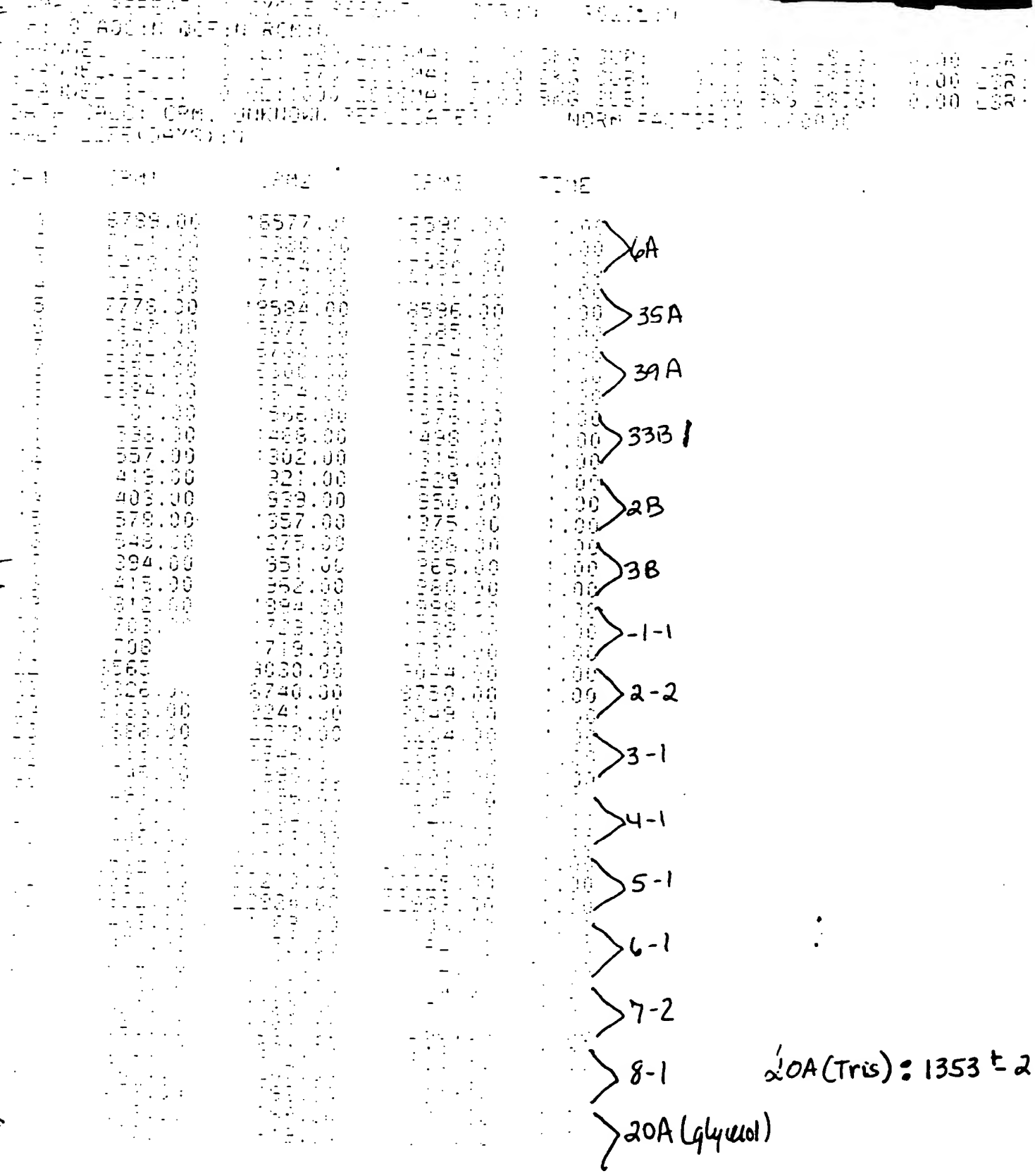
EXHIBIT PAGE #18

1 ug/assay NAD glycohydrolase

20A (glycuol)	805 ± 12	Net cpm	pmoles rel/min/ug
6A	17,310 ± 701	16505	4.02
35A	18,257 ± 1023	17452	4.25
39A	6645 ± 309	5840	1.42
33B	1452 ± 136	647	0.15
2B	1072 ± 248	267	0.065
3B	1062 ± 184	257	0.062
20A (TMG)	1355 ± 278	—	—
1-1	1814 ± 156	1009	0.24
2-2	8670 ± 399	7865	1.9
3-1	2303 ± 329	1498	6.36
4-1	1279 ± 87	372	0.09
5-1	22,615 ± 796	21,810	5.3
6-1	1085 ± 70	280	0.068
7-2	1169 ± 102	364	0.088
8-1	1233 ± 59	428	0.10

EXHIBIT NO. #19

[redacted]



Need ADP-ribosyltransferase

EXHIBIT PAG = -

NAD glycohydrolase Activity – Arrayed mutants (New pipetting Tris buffer)

2 hrs, 30°C, 30uM NAD

CPM (TOTAL)

Construct	0.25 ug	0.5 ug	1.0 ug
purified SI from PTx	6,340 (1.54)	12,980.5 (3.16)	—
20A	FZB		-23 (0)
6A	1480.5 (0.36)	3,074 (0.75)	6446 (1.57)
1-1	73.5 (0.02)	254.5 (0.06)	486.5 (0.12)
2-2	562.5 (0.14)	1340 (0.33)	2734 (0.66)
3-1	125 (0.03)	419 (0.10)	882.5 (0.21)
4-1	31.5 (0.008)	-11 (0) (0)	34.5 (0.008)
5-1	1369 (0.33)	3011 (0.73)	6204 (1.51)
6-1	-5 0	-42 0	-30.5 0
7-2	-59 0	15 0	-58 0
8-1	-5.5 0	-4 0	204 (0.05)
81/1-4	-60 0	-99.5 0	-64 0

cpm x 1.5 (ml) x 1.5 (moles) x 1.3 ÷ 100 ÷ 120 (hrs) ÷ ug

= .0022437 ÷ ug = umoles/min.

EXHIBIT PAGE #1

[redacted]

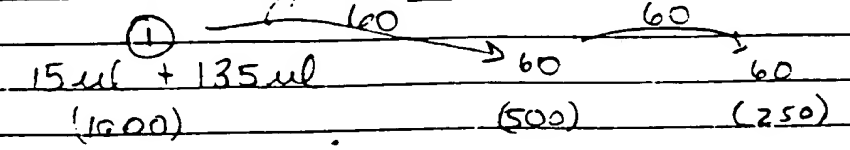
SAM	CPM1	CPM2	CPM3	TIME	
	253.00	533.00	533.00	1.00	> Bkgd.
	381.00	751.00	775.00	1.00	
	1429.00	2445.00	2453.00	1.00	
	497.00	3554.00	3573.00	1.00	
	5456.00	13055.00	13052.00	1.00	> 5I 0.5
	5963.00	14300.00	14314.00	1.00	
	2780.00	6637.00	6647.00	1.00	> " 0.25
	3170.00	7481.00	7492.00	1.00	
	3777.00	7116.00	7122.00	1.00	> 6A 1.0
	3851.00	7170.00	7178.00	1.00	
	1510.00	3808.00	3822.00	1.00	> " 0.5
	1553.00	3734.00	3745.00	1.00	
	956.00	2258.00	2266.00	1.00	> " 0.25
	879.00	2097.00	2104.00	1.00	
	432.00	1096.00	1104.00	1.00	> 1-1 1.0
	523.00	1271.00	1286.00	1.00	
	435.00	978.00	991.00	1.00	> " 0.5
	344.00	925.00	933.00	1.00	
	322.00	760.00	778.00	1.00	> " 0.25
	315.00	781.00	791.00	1.00	
	1299.00	3243.00	3250.00	1.00	> 2-2 1.0
	1449.00	3619.00	3630.00	1.00	
	802.00	1999.00	2009.00	1.00	> " 1.0
	819.00	2079.00	2082.00	1.00	
	[illegible]	[illegible]	[illegible]	1.00	> " 1.0
	[illegible]	[illegible]	[illegible]	1.00	
	548.00	1254.00	1270.00	1.00	
	[illegible]	[illegible]	1545.00	1.00	> 3-1 1.0
	[illegible]	[illegible]	[illegible]	1.00	
	[illegible]	[illegible]	1476.00	1.00	> " 0.5
	[illegible]	[illegible]	[illegible]	1.00	
	[illegible]	[illegible]	941.00	1.00	> " 0.25
	[illegible]	[illegible]	[illegible]	1.00	
	[illegible]	[illegible]	[illegible]	1.00	> 4-1 1.0
	[illegible]	[illegible]	[illegible]	1.00	
	[illegible]	[illegible]	[illegible]	1.00	> " 0.5
	[illegible]	[illegible]	[illegible]	1.00	
	[illegible]	[illegible]	[illegible]	1.00	> " 0.25
	[illegible]	[illegible]	[illegible]	1.00	
	[illegible]	[illegible]	1625.00	1.00	> 5-1 1.0
	[illegible]	[illegible]	[illegible]	1.00	
	[illegible]	[illegible]	[illegible]	1.00	> " 0.5
	[illegible]	[illegible]	[illegible]	1.00	
	[illegible]	[illegible]	[illegible]	1.00	> " 0.25

EXHIBIT F.6E -

[redacted]

NPO glycohydrolase Activity Assay: matrix, ~~Capsicum~~ Capsicum at 250, 500 and 1000 mg 2 hour 30°C

PSI (4105 ug/ml)
dilute to 20 ug/ml at 1:10 and 50 ul for each pt;



Nutmits: at 75 ug/ml

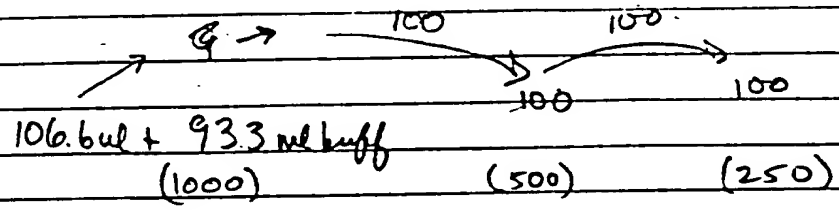


EXHIBIT PAGE 2 -

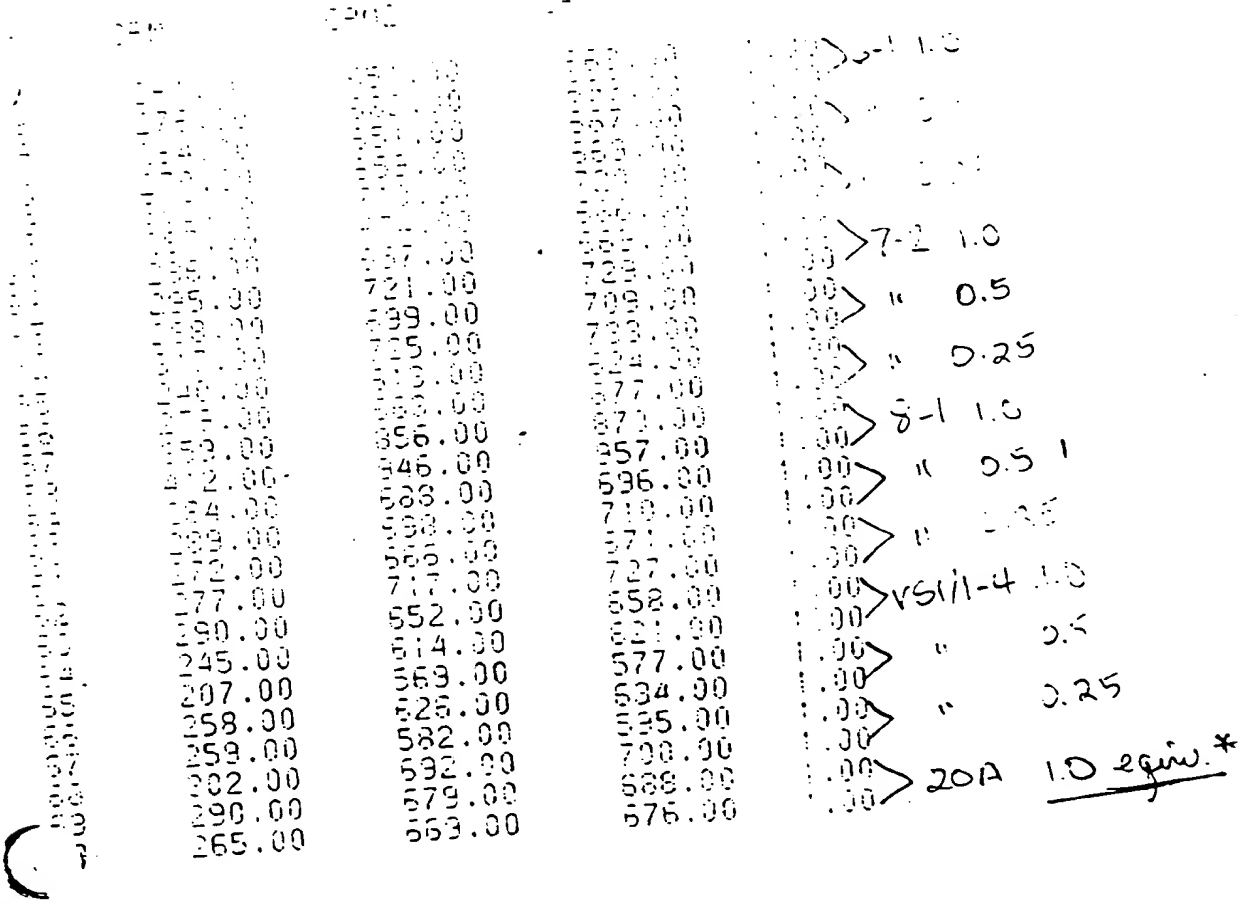


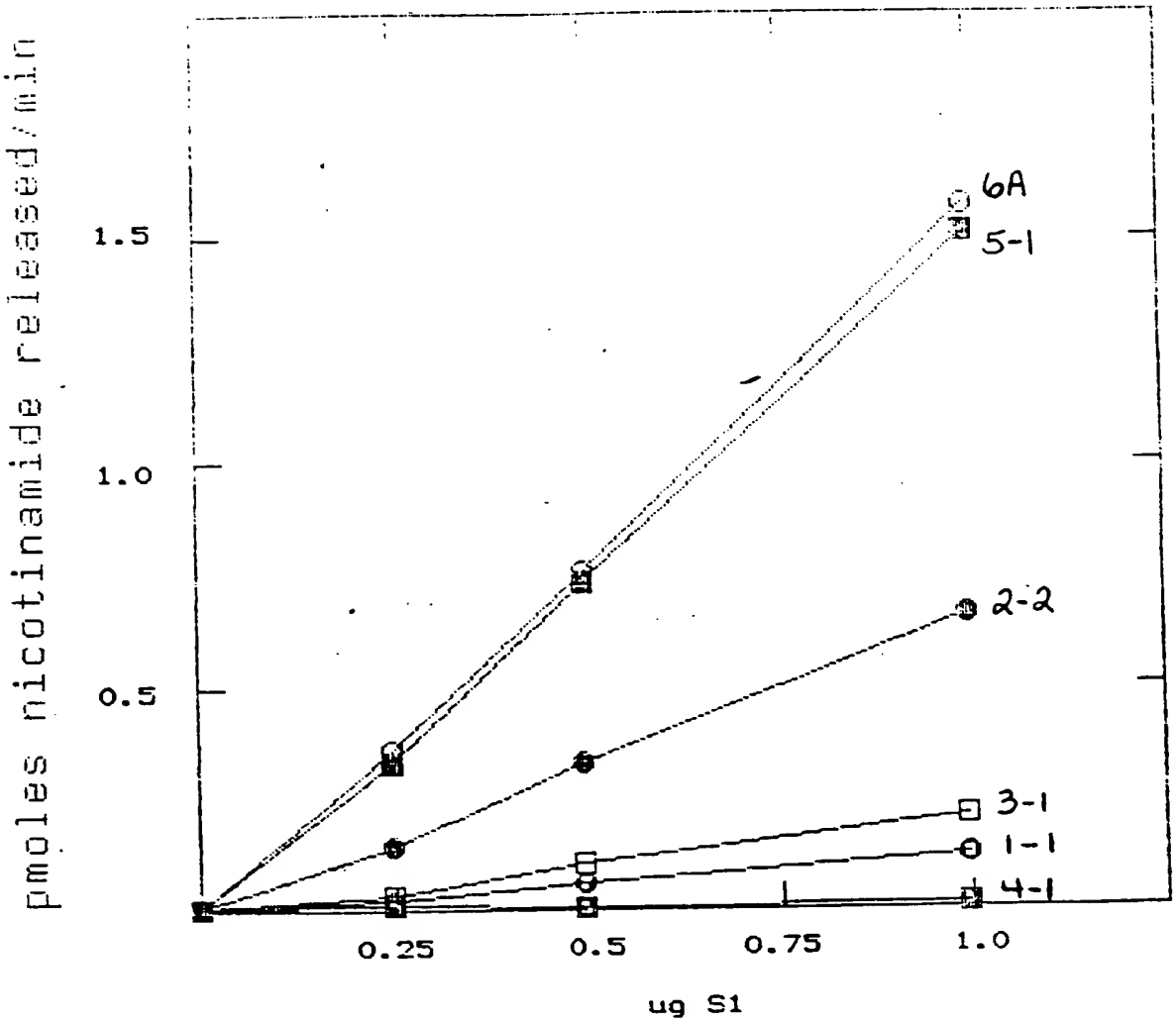
EXHIBIT PAGE # 24

[redacted]

	0.25	0.5	1.0	% carbol
½A	0.36	0.75	1.57	100
1-1	0.02	0.06	0.12	7.6
2-2	0.14	0.33	0.66	42.0
3-1	0.03	0.10	0.21	13.3
4-1	0.008	0.0	0.008	0.51
5-1	0.33	0.73	1.51	96.7
6-1		0	0	0
7-2		0	0.05	3.1
8-1		0	0	0

NAD Glycohydrolase Activity

EXHIBIT PAGE #25



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket N . 40399/177/NIHD

In re patent application of

Jerry M. Keith

Serial No. 07/542,149 Group Art Unit: 1814

Filed: June 22, 1990 Examiner: G. Bugaisky

For: PERTUSSIS TOXIN GENE: CLONING AND EXPRESSION

DECLARATION OF WITOLD CIEPLAK, JR.

The Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Sir:

I, Witold Cieplak, Jr. hereby declare that:

1. I previously executed a declaration for this application. In my previous declaration, I stated that I carried out the experiments recorded on notebook pages attached to a declaration by Dr. Jerry Keith. A copy of that declaration by Dr. Keith was attached to my previous declaration as Appendix 1. With the exception of the notations on the top of each page regarding exhibit page numbers, the handwriting on all of those notebook pages is my handwriting.

2. At the time I performed those experiments, it was my practice to record my notes in a looseleaf notebook. Hence, there is no notebook cover bearing my name or table of contents page reflecting those experiments.

3. During the course of my research at Rocky Mountain Laboratories, NIAID (Hamilton, Montana), I conceived that a mutation at the arginine 9 position of the amino acid sequence of the S1 subunit of *Bordetella pertussis* toxin could yield a substantially detoxified mutant comprising an epitope that contributes to

immunoprotection against *Bordetella pertussis* toxicity. I subsequently discovered that such a mutation at the arginine 9 position in fact yielded a substantially detoxified mutant comprising an epitope that contributes to immunoprotection against *Bordetella pertussis* toxicity.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent resulting therefrom.

12/11/93
Date

Witold Ciepiak, Jr.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No. 40399/177/NIHD

In re patent application of

Jerry M. Keith

Serial No. 07/542,149 Group Art Unit: 1814

Filed: June 22, 1990 Examiner: G. Bugaisky

For: PERTUSSIS TOXIN GENE:
CLONING AND EXPRESSION

DECLARATION OF JERRY M. KEITH

The Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Sir:

I, Jerry M. Keith, hereby declare that:

1. I have reviewed the Declaration of Dr. Cieplak attached hereto. I believe all statements in that declaration to be correct.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent resulting therefrom.

December 21, 1993 Jerry M. Keith

Date Jerry M. Keith

Claims 11, 13 and 15-16 are allowable. Prosecution is now closed.

The amendment to the specification is entered, as it is clear that an inadvertent error in sequencing of the deposited parental strain occurred. The amendment does not constitute new matter.

The change in inventorship is permissible. It does not appear necessary to revive parent application 07/311,612 in order to grant priority (MPEP § 201.3 re continuing applications). There is, however, now no continuity between this application and 06/843,727 (Patent No. 4,883,761).

All claims are allowable. However, due to a potential interference, *ex parte* prosecution is SUSPENDED FOR A PERIOD OF 3 MONTHS FROM THE DATE OF THIS LETTER.

Upon expiration of the period of suspension, applicant should make an inquiry as to the status of the application.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Gabriele E. Bugaisky, Ph.D. whose telephone number is (703) 308-4201.

Papers related to this application may be submitted to Group 180 by facsimile transmission. Papers should be faxed to Group 180 via the PTO Fax Center located in Crystal Mall 1. The faxing of such papers must conform with the notice published in the Official Gazette, 1096 OG 30 (November 15, 1989). The CM-1 Fax Center numbers are (703) 308-4227 and (703) 305-3014.

Any inquiry of a general nature or relating to the status of this application should be directed to the Group receptionist whose telephone number is (703) 308-0196.

ROBERT A. WAX
SUPERVISORY PATENT EXAMINER
GROUP 180

geb
April 27, 1994

Current counts of TCA pellets.

PAGE

USER: 2 ID:SURVEY PRESET TIME: 1.00
SAMPLE REPEAT:T CYCLE REPEAT:1 SCR:N RS232:N
H#: 0 AQC:N QDE:N RCM:N
CHANNEL 1-LL: 0 UL: 400 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR:
CHANNEL 2-LL: 0 UL: 670 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR:
CHANNEL 3-LL: 0 UL:1000 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR:
DATA CALC: CTS, UNKNOWN REPLICAIES: 1 NORM FACTOR:Q 1.00000
HALF-LIFE(DAYS):N

SAM	CTS1	CTS2	CTS3	TIME	
1	51.00	62.00	62.00	1.00	Buffer
2	3968.00	4089.00	4089.00	1.00	PIX (0.1 ug)
3	315.00	347.00	347.00	1.00	S13 supt (8 ug)
4	3560.00	3670.00	3670.00	1.00	S13 pellet (8 ug)
5	471.00	494.00	494.00	1.00	S11 supt (8 ug)
6	709.00	729.00	729.00	1.00	S11 pellet (8 ug)
7	2857.00	2950.00	2950.00	1.00	6A-4 #2
8	3071.00	3157.00	3157.00	1.00	6A-4 #4
9	2354.00	2431.00	2431.00	1.00	6A-4 #6
10	2271.00	2361.00	2361.00	1.00	6A-4 #8
11	2126.00	2161.00	2161.00	1.00	Aargn rSi (0.8 ug)
12	2982.00	3086.00	3086.00	1.00	6A-3
13	3195.00	3294.00	3294.00	1.00	35A
14	1253.00	1298.00	1298.00	1.00	39A
15	110.00	120.00	120.00	1.00	33B
16	76.00	89.00	89.00	1.00	2B
17	184.00	195.00	195.00	1.00	3B
18	87.00	97.00	97.00	1.00	14B
19	249.00	258.00	258.00	1.00	21B ?
20	309.00	322.00	322.00	1.00	43B ?
21	718.00	755.00	755.00	1.00	1-1
22	2087.00	2170.00	2170.00	1.00	2-2
23	781.00	818.00	818.00	1.00	3-1
24	73.00	85.00	85.00	1.00	4-1
25	3334.00	3458.00	3458.00	1.00	5-1
26	395.00	405.00	405.00	1.00	6-1 ?
27	407.00	418.00	418.00	1.00	6A-2 ?
28	372.00	377.00	377.00	1.00	7-2 ?
29	789.00	803.00	803.00	1.00	8-1 ?

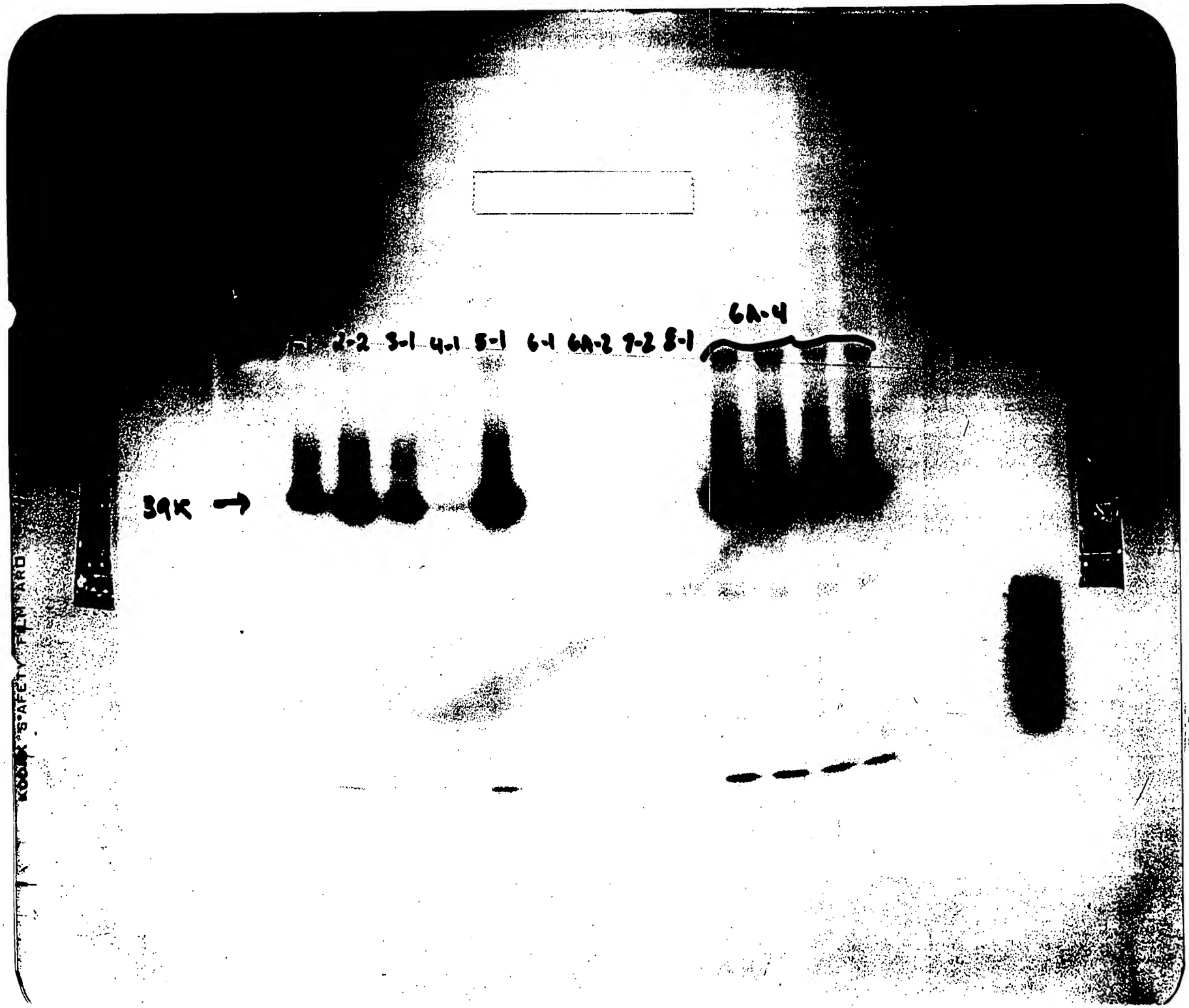
6A-4 #2, #4, #6, #8: 2 ug
all 2 ug

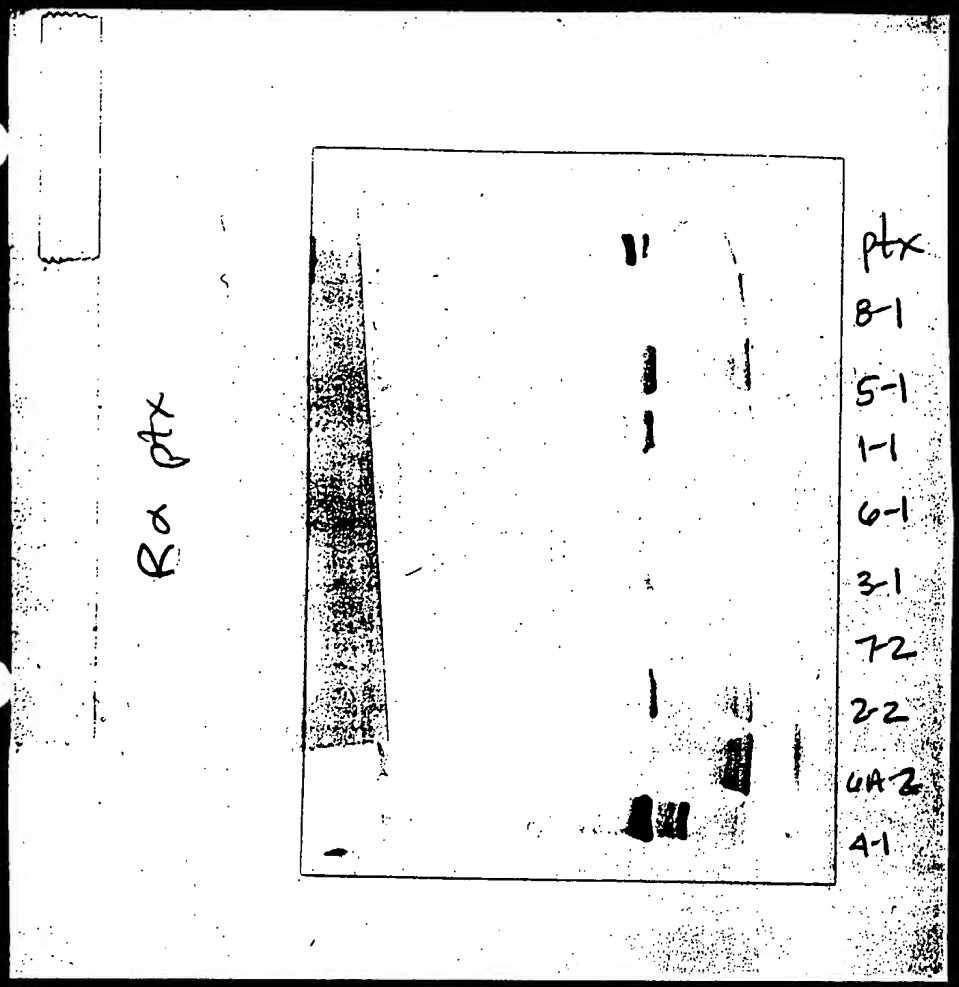
Assay: 10 ul 4X ADPR cocktail
20 ul test sample (100 ug/ml - 400 ug/ml)
10 ul Transducin (~0.7 ug)
Stock
↓
0.5 HR 37°C
↓
1 ul 20 mg/ml BSA (next use OVA + 50 ul in 10 ml of 1-5 mg/ml)
↓
50 ul ice cold 10% TCA
↓ O.N. 4°
↓
1 ml 5% TCA x 2

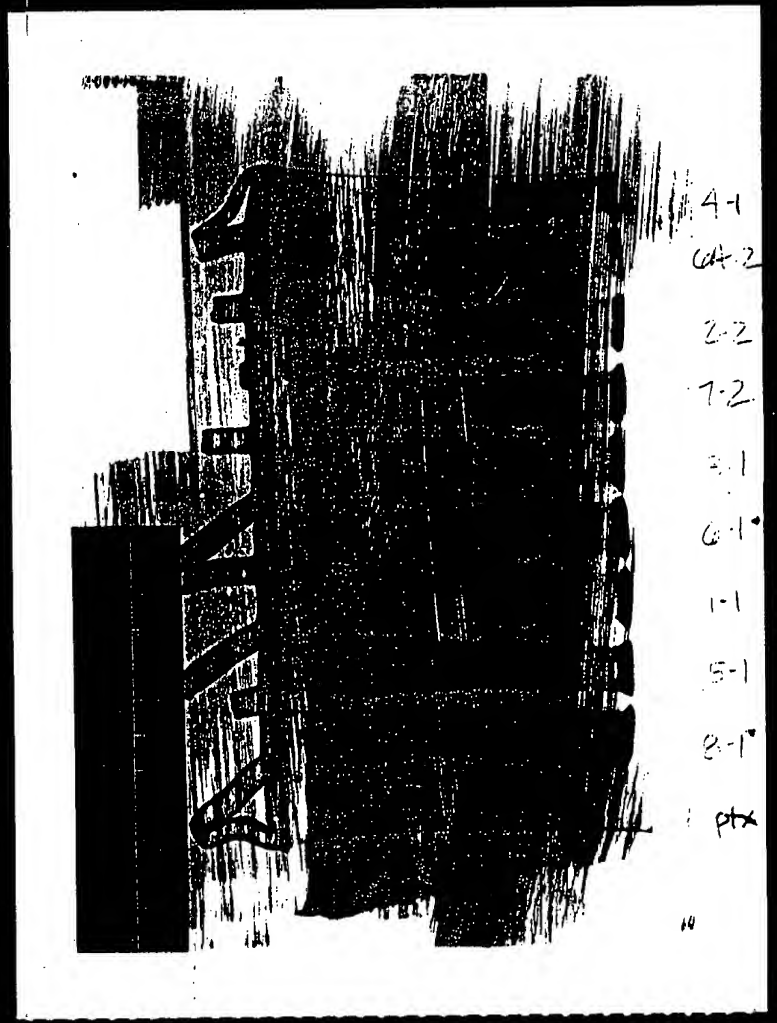
1 ml Ether
aspirate to dryness count

ADP-ribosyl transferase
2nd gel -

Lane	
1	~~Blank~~
2	Bio-Rad
3	1-1
4	2-2
5	3-1
6	4-1
7	5-1
8	6-1
9	6A-2
10	7-2
11	81
12	6A-4 #2
13	6A-4 #4
14	6A-4 #6
~~15~~	6A-4 #8
15	


2-2
3-1
4-1
5-1
6-1
6A-2
7-2
8-1
6A-4
39K
KODAK "S" SAFETY FILM CARD


Rα ptx
ptx
8-1
5-1
1-1
6-1
3-1
7-2
2-2
GA-2
4-1


4-1
GA-2
2-2
7-2
3-1
6-1
1-1
5-1
8-1
ptx

← this is the band

TABLE 2

Complete Nucleotide Sequence of Pertussis Toxin Gene

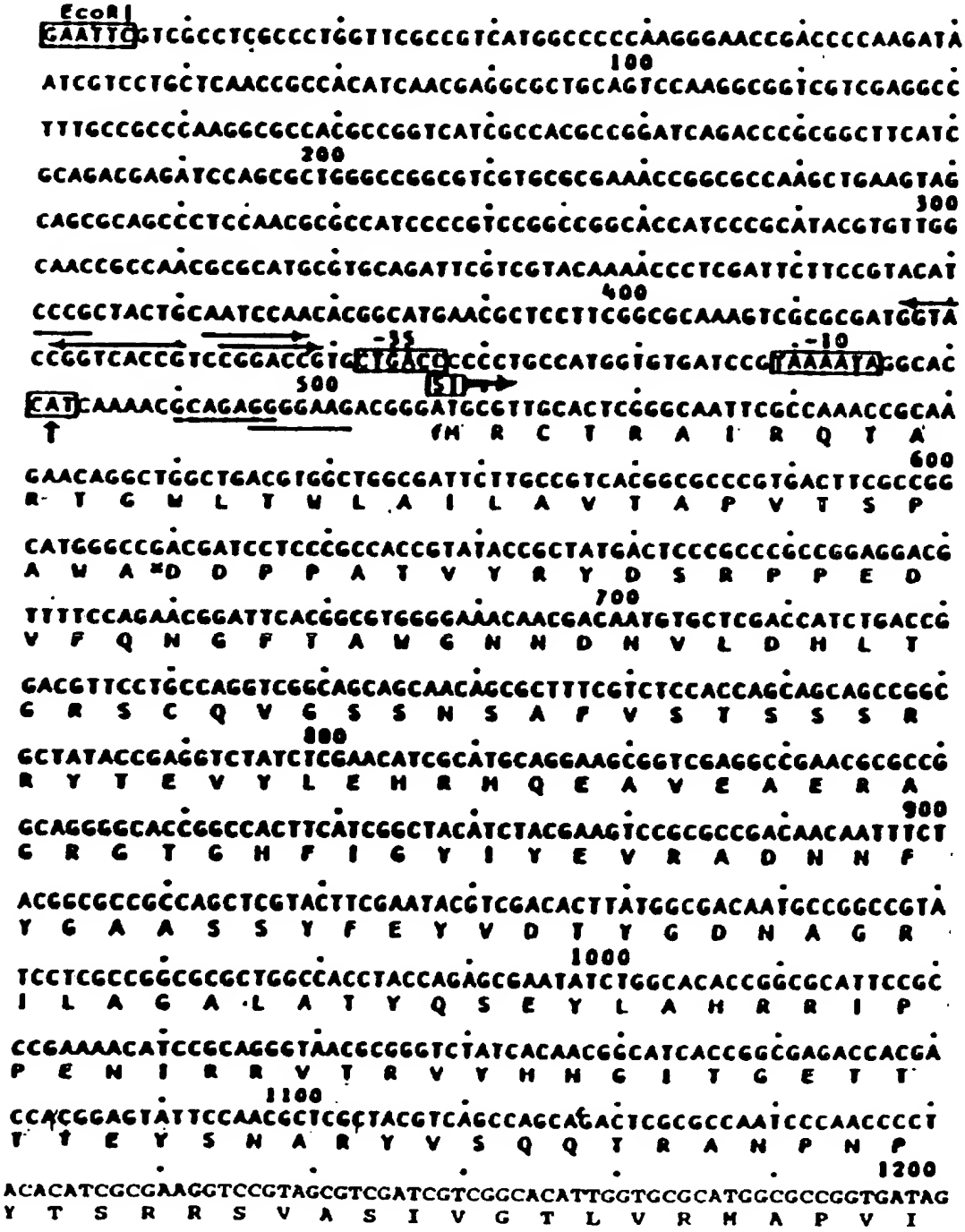


TABLE 2

Complete Nucleotide Sequence of Pertussis Toxin Gene

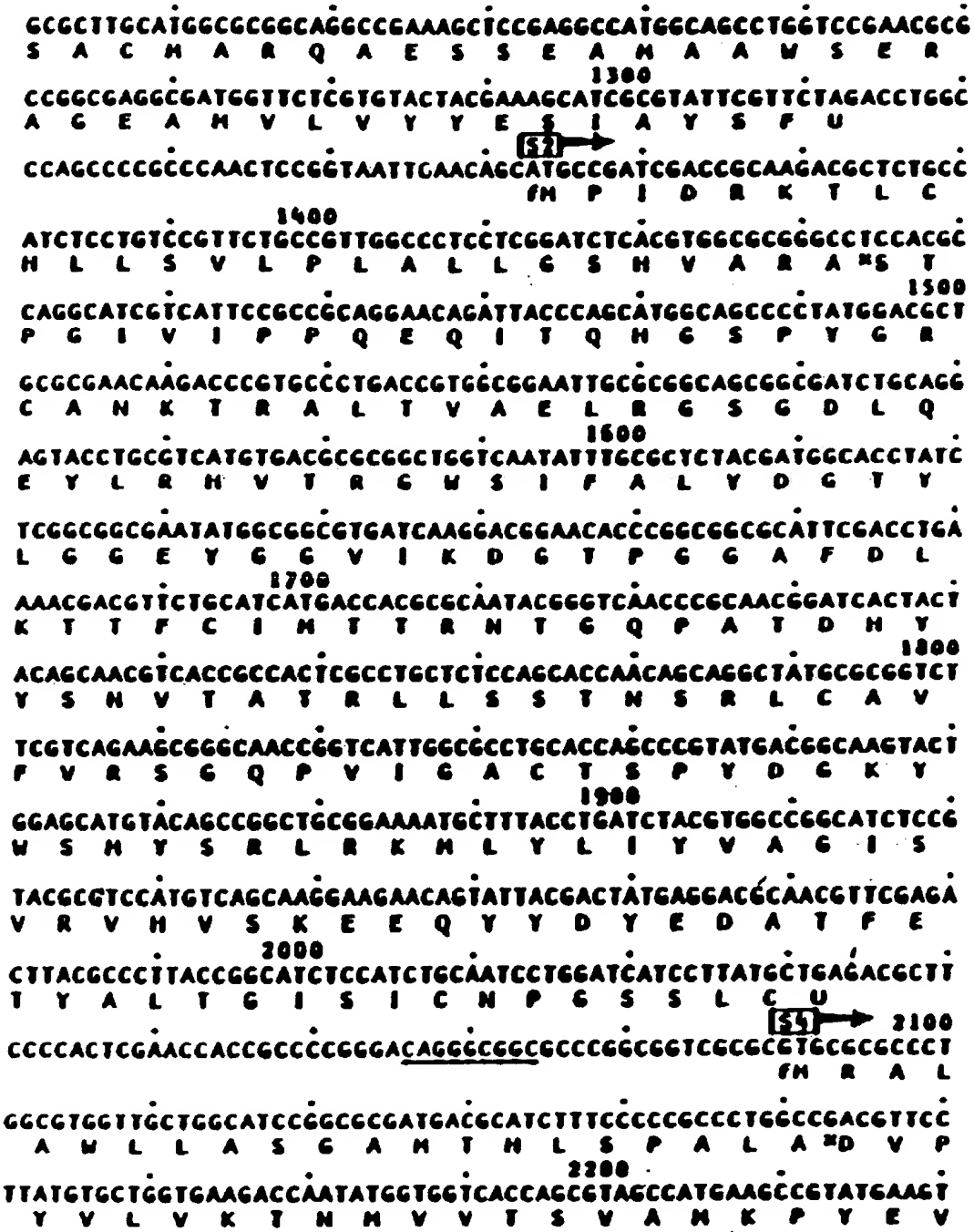


TABLE 2

Complete Nucleotide Sequence of Pertussis Toxin Gene

```
CACCCCGACGCGCATGCTGGTCTGCGGCATCGCCGCCAAACTGGGCGCCGCGGCCAGCAG
  T  P  T  R  M  L  V  C  G  I  A  A  K  L  G  A  A  A  S  S
                    2300
CCCGGACGCGCACGTGCCGTTCTGCTTCGGCAAGGATCTCAAGCGTCCCGGCAGCAGTCC
  P  D  A  H  V  P  F  C  F  G  K  D  L  K  R  P  G  S  S  P
                                                    2400
CATGGAAGTCATGTTGCGCGCCGTCTTCATGCAACAACGGCCGCTGCGCATGTTTCTGGG
  M  E  V  M  L  R  A  V  F  M  Q  Q  R  P  L  R  M  F  L  G

TCCCAAGCAACTCACTTTCGAAGGCAAGCCCGCGCTCGAACTGATCCGGATGGTCGAATG
  P  K  Q  L  T  F  E  G  K  P  A  L  E  L  I  R  M  V  E  C
                                   S5 →
CAGCGGCAAGCAGGATTGCCCCTGAAGGCGAACCCCATGCATACCATCGCATCCATCCTG
  S  G  K  Q  D  C  P  U           fM  H  T  I  A  S  I  L

TTGTCCGTGCTCGGCATATACAGCCCGGCTGACGTCGCCGGCTTGCCGACCCATCTGTAC
  L  S  V  L  G  I  Y  S  P  A  D  V  *A  G  L  P  T  H  L  Y
                    2600
AAGAACTTCACTGTCCAGGAGCTGGCCTTGAAACTGAAGGGCAAGAATCAGGAGTTCTGC
  K  N  F  T  V  Q  E  L  A  L  K  L  K  G  K  N  Q  E  F  C
                                                    2700
CTGACCGCCTTCATGTCGGGCAGAAGCCTGGTCCGGGCGTGCCTGTCCGACGCGGGACAC
  L  T  A  F  M  S  G  R  S  L  V  R  A  C  L  S  D  A  G  H

GAGCACGACACGTGGTTCGACACCATGCTTGGCTTTGCCATATCCGCGTATGCGCTCAAG
  E  H  D  T  W  F  D  T  M  L  G  F  A  I  S  A  Y  A  L  K
                                   2800
AGCCGGATCGCGCTGACGGTGGAAGACTCGCCGTATCCGGGCACTCCCGGCGATCTGCTC
  S  R  I  A  L  T  V  E  D  S  P  Y  P  G  T  P  G  D  L  L

GAACTGCAGATCTGCCCGCTCAACGGATATTGCGAATGAACCCTTCCGGAGGTTTCGACG
  E  L  Q  I  C  P  L  N  G  Y  C  E  U
                    2900
TTTCCGCGCAATCCGCTTGAGACGATCTTCCGCCCTGGTTCCATTCCGGGAACACCGCAA
                                                    3000
S3 →
CATGCTGATCAACAACAAGAAGCTGCTTCATCACATTCTGCCCATCCTGGTGCTCGCCCT
  fM  L  I  N  N  K  K  L  L  H  H  I  L  P  I  L  V  L  A  L

GCTGGGCATGCGCACGGCCCAGGCCGTTGCGCCAGGCATCGTCATCCCGCCGAAGGCACT
  L  G  M  R  T  A  Q  A  *V  A  P  G  I  V  I  P  P  K  A  L
                                   3100
GTTCACCCAACAGGGCGGCGCCTATGGACGCTGCCCGAACGGAACCCGCGCCTTGACCGT
  F  T  Q  Q  G  G  A  Y  G  R  C  P  N  G  T  R  A  L  T  V

GGCCGAACTGCGCGGCAACGCCGAATTGCAGACGTATTTGCGCCAGATAACGCCCGGCTG
  A  E  L  R  G  N  A  E  L  Q  T  Y  L  R  Q  I  T  P  G  W
                    3200
GTCCATATACGGTCTCTATGACGGTACGTACCTGGGCCAGGCGTACGGCGGCATCATCAA
  S  I  Y  G  L  Y  D  G  T  Y  L  G  Q  A  Y  G  G  I  I  K
                                                    3300
GGACGCGCCGCCAGGCGCGGGGTTCATTTATCGCGAAACTTTCTGCATCACGACCATATA
  D  A  P  P  G  A  G  F  I  Y  R  E  T  F  C  I  T  T  I  Y
```

TABLE 2

Complete Nucleotide Sequence of Pertussis Toxin Gene

```
CAAGACCGGGCAACCGGCTGCGGATCACTACTACAGCAAGGTCACGGCCACGCGCCTGCT
  K  T  G  Q  P  A  A  D  H  Y  Y  S  K  V  T  A  T  R  L  L
                                        3400
CGCCAGCACCAACAGCAGGCTGTGCGCGGTATTCGTCAGGGACGGGCAATCGGTCATCGG
  A  S  T  N  S  R  L  C  A  V  F  V  R  D  G  Q  S  V  I  G

AGCCTGCGCCAGCCCGTATGAAGGCAGGTACAGAGACATGTACGACGCGCTGCGGCGCCT
  A  C  A  S  P  Y  E  G  R  Y  R  D  M  Y  D  A  L  R  R  L
                 3500
GCTGTACATGATCTATATGTCCGGCCTTGCCGTACGCGTCCACGTCAGCAAGGAAGAGCA
  L  Y  M  I  Y  M  S  G  L  A  V  R  V  H  V  S  K  E  E  Q
                                                       3600
GTATTACGACTACGAGGACGCCACATTCCAGACCTATGCCCTCACCGGCATTTCCCTCTG
  Y  Y  D  Y  E  D  A  T  F  Q  T  Y  A  L  T  G  I  S  L  C

CAACCCGGCAGCGTCGATATGCTGAGCCGCCGGCTCGGATCTGTTCGCCTGTCCATGTTT
  N  P  A  A  S  I  C  U
                                        3700
TTCCTTGACGGATACCGCGAATGAATCCCTTGAAAGACTTGAGAGCATCGCTACCGCGCC

TGGCCTTCATGGCAGCCTGCACCCTGTTGTCCGCCACGCTGCCCGACCTCGCCCAGGCCG
                 3800
GCGGCGGGCTGCAGCGCTGTCAACCACTTCATGGCGAGCATCGTGGTCGTACTGCCGCGG
                                                       3900
CGGTCAGTGGCCACGGTGACCATCGCCATAATCTGGGCGGGCTACAAGCTGCTGTTCCGG

CACGCCGATGTGCTGGACGTGGTGCGTGTGGTGCTGGCGGGAGCTGCTGATCGGCGCATC
                                        4000
GGCCGAAATCGCTCGTTATCTGCTGACCTGAATCCTGGACGTATCGAACATGCGTGATCC
                                                  ←
GCTTTTCAAGGGCTGCACCCGGCGCCGCGATGCTGATGGCGTACCCGCCACGGCAGGCCG
                 4100                                  →
TGTGCAGCCGGCACCATTCCCTGCTGGGCCATCTCGGTTCAGCATCCGCTTTCTGGCCTT
                                                       4200
GTTTCCCGTGGCATTGCTGGCGATGCGGATCATGATCCGGCGCGATGACCAGCAGTTCCG
  Sau3A
CCTGATC
```

The deduced amino acid sequences of the individual subunits are shown in the single letter code below the nucleotide sequence. The proposed signal peptide cleavage sites are indicated by asterisks. The start of the protein coding region for each subunit is indicated by the box and arrow over the initiation codon. Putative ribosomal binding sites are underlined. The promotor-like sequence is shown in the -35 and -10 boxes. Proposed transcriptional start site is indicated by th arrow in the CAT box. Inv rted repeats are indicated by the arrows in the flanking r gions.

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.